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Original Article

# Mechanistic and phenotypic studies of bicarinalin, BP100 and colistin action on *Acinetobacter baumannii*

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ABSTRACT

*Acinetobacter baumannii* has been identified by the WHO as a high priority pathogen. It can be resistant to multiple antibiotics and colistin sulphate is often used as a last-resort treatment. However, the potentially severe side-effects of colistin are well documented and this study compared the bactericidal and anti-biofilm activity of two synthetic nature-inspired antimicrobial peptides, bicarinalin and BP100, with colistin. The minimum bactericidal concentration (MBC) against planktonic *A. baumannii* was approximately 0.5 μg/ml for colistin sulphate and ~4 μg/ml for bicarinalin and BP100. *A. baumannii* commonly occurs as a biofilm and biofilm removal assay results highlighted that both bicarinalin and BP100 had significantly greater potential than colistin. Atomic force microscopy (AFM) showed dramatic changes in *A. baumannii* cell size and surface conformity when treated with peptide concentrations at and above the MBC. Scanning electron microscopy (SEM) visualised the reduction of biofilm coverage and cell surface changes as peptide concentration increased. Liposome assays revealed that these peptides most likely act as pore-forming agents in the membrane. Bicarinalin and BP100 may be effective therapeutic alternatives to colistin against *A. baumannii* infections but further research is required to assess if they elicit cytotoxicity issues in patients.



## 1. Introduction

It has been widely publicised that by 2050, deaths due to antimicrobial resistant (AMR) infections may rise to 10 million per year and during that time, 300 million people will die from AMR infections [1]. The abuse of currently used antimicrobials, the paucity of new antimicrobials progressing successfully through clinical trials and the evolution of bacterial resistance mechanisms have led to the prospect of returning to the pre-antibiotic era when patients were dying from infections through minor injuries and routine surgeries. Although the recent development of novel antibiotics [2,3] has provided reason for optimism, these drugs are generally limited to the treatment of Gram-positive infections while Gram-negative pathogens remain of significant concern [4].

*Acinetobacter baumannii* is one such opportunistic pathogen [5,6] occurring almost exclusively in the hospital environment and is particularly prevalent in intensive care and burns units; it has been reported to be responsible for between 2 and 10% of all Gram-negative nosocomial infections [7]. A member of the 'ESKAPE' group [8] of pathogens, *A. baumannii* is able to persist on clinical surfaces by forming biofilms [7,9], an ability that renders bacteria more resistant to many common antimicrobials [10,11] leading to *A. baumannii* infections becoming rapidly more difficult to treat [12]. It is able to persist for long periods on fomites [7,9] enabling the pathogen to cause widespread epidemic infections in nosocomial settings. Current treatments include β-lactam antibiotics with the carbapenems typically the treatment of choice [9].

Antimicrobial peptides (AMPs) have been much heralded as alternatives to antibiotics due to their ability to destroy multi-drug resistant (MDR) bacteria [13]. One example is colistin sulphate (colistin), commonly used as a last resort treatment for MDR *A. baumannii* infections [14]. However, the toxicity of colistin in patients is well known [13,14] and resistance is increasingly described [15–17] resulting in a crucial search for improved alternatives.

The aim of this study was to establish and visualise the activity of two little researched AMPs, bicarinalin and BP100, which may be appropriate for use instead of colistin to treat MDR *A. baumannii* infections. Bicarinalin is an amphipathic, C-terminally amidated, novel antimicrobial peptide derived from the venom of the myrmicine ant, *Tetramorium bicarinatum* [18,19]. It consists of a sequence of 20 amino acid residues (KIKIPWGKVKDFLVGGMKAV) with a molecular weight of 2213.78 g/mol. The action of bicarinalin has been evaluated against a selection of Gram-positive and Gram-negative or-

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ganisms [18,19] and has been found to have good antibacterial activity compared to other AMPs together with less haemolytic activity.

BP100 is a short C-amidated undecapeptide consisting of 11 amino acids (KKLFKKILKYL) and a molecular weight of 1420.88 g/mol. It was originally synthesised by combinatorial chemistry involving two peptides, cecropin A and melittin [20]. Cecropin A is a member of the well-researched AMP family, the cecropins, first isolated from the giant silk moth *Hyalophora cecropia*. The cecropin family, although susceptible to protease degradation, does not exhibit cytotoxic effects against human erythrocytes [21,22]. Melittin is a 26 amino acid, haemolytic alpha helical peptide first purified from the European honeybee in 1958 [23], with demonstrated antibacterial activity [24]. However, on its own, melittin's strong cytotoxic action makes it unsuitable for clinical applications [25]. To circumvent cecropin A's susceptibility to proteolytic degradation and melittin's high cytotoxicity, they were combined to produce a derivative, BP100. BP100 exhibits low susceptibility to protease degradation and lower cytotoxicity against erythrocytes and fibroblasts [26]. BP100 has been established to have good antibacterial activity against several Gram-negative bacteria [20,22].

It is believed that bicarinalin's antibacterial mechanism is similar to other AMPs, with its cationic charge, as a result of lysine residues, naturally attracted to the anionic charged bacterial cell surface. It is also believed that BP100 interacts with the bacterial cell membrane via electrostatic attraction to the negatively charged LPS layer, causing blebbing on the surface leading to a collapse of the outer membrane [20].

The aim of this study was to assess the potential of bicarinalin and BP100 as alternatives to colistin to treat *A. baumannii* infections. The study also aimed to visualise the effects of these antimicrobial agents on *A. baumannii* cells and biofilms using scanning electron microscopy (SEM) and atomic force microscopy (AFM).

## 2. Materials and methods

### *2.1. Bacterial culture conditions*

Fresh cultures of *A. baumannii* (ATCC® 19,606) were prepared by streaking a culti-loop (ThermoFisher Scientific, UK) on freshly prepared Tryptone Soya Agar (TSA, Oxoid, UK) and incubated for 24 h at 37 °C. Purity was assessed using Gram staining, cell morphology, oxidase and Analytical Profile Index (API, Biomérieux, UK, 20NE kit) testing. A standard growth curve for the *A. baumannii* was established to ensure mid-log phase growth and an initial inoculum of $5 \times 10^5$ CFU/ml at the start of each experiment.

### *2.2. Antimicrobial assays*

#### *2.2.1. Minimum inhibitory concentration (MIC)*

The MIC was determined for each of the antimicrobial agents studied: colistin (Sigma–Aldrich, UK), bicarinalin (97.7% purity, Genscript, USA) and BP100 (98.4% purity, Genscript, USA). 10 ml of sterile Tryptone Soya Broth (TSB, Oxoid, UK) was inoculated with 2–3 colonies of *A. baumannii* and incubated overnight at 37 °C and later diluted to an absorbance that equated to $1 \times 10^6$ CFU/ml.

Antimicrobial solutions were prepared and sterilised by filtering through a 0.2 μm minisart single use sterile filter (Sartorius-Stedim Biotech, Fisherscientific, UK). Stock solutions (1024 μg/ml) were prepared, taking into account the stated product purity. Reduction of peptide concentration through filtration was not assayed for.

50 μl of each agent, at a stock concentration of 1024 μg/ml, was added to well 1 of a 96-well microplate (Fisherscientific, UK) and a two-fold dilution series prepared in wells 2 to 12. 50 μl of inoculated TSB, containing $1 \times 10^6$ CFU/ml of *A. baumannii*, was then added to each well resulting in final peptide concentrations from 256 μg/ml to 0.125 μg/ml and a cell density of $5 \times 10^5$ CFU/ml. Plates were covered and incubated in a shaking incubator at 37 °C and 140 rpm for 24 h. The lowest concentration of peptide where the well was visibly clear was recorded as the MIC. The entire experiment was carried out three times in triplicate to give nine datasets and the mean MIC established.

#### *2.2.2. Minimum bactericidal concentration (MBC)*

After recording the MIC, spread plates were prepared on TSA using 100 μl from each clear well. These plates were incubated at 37 °C for 24 h. The lowest concentration where there was no growth observed on the plate was recorded as the MBC. This was carried out after each MIC test and therefore three times in triplicate.

#### *2.2.3. Minimum biofilm inhibitory concentration (MBIC)*

A 96-well microplate plate containing doubling dilutions of antimicrobial was incubated with $5 \times 10^5$ CFU/ml *A. baumannii* for 24 h at 37 °C, 140 rpm. After 24 h, the wells were emptied and washed three times with ¼ strength Ringer's solution and air dried for 1 h. 1% crystal violet solution was added to each well and left at room temperature for 10 min. The wells were emptied by pipetting, washed three times with distilled water, and air dried for 30 min at 37 °C. The stain was solubilised with 96% ethanol (100 μl). The plate was covered and shaken at 140 rpm for 30 min 2 μl was removed and the absorbance of the solution was measured at 590 nm compared to a 96% ethanol blank. This was replicated three times and the mean calculated. The percentage biofilm inhibited was calculated by comparing against an untreated bacterial control. SPSS Statistics 21 (IBM, USA) was used for the statistical analysis of MBIC results. The samples t-test was used to find if there was a statistical difference of 95% ($p \leq 0.05$, statistically significant) or 99% ($p \leq 0.01$, highly significant).

### *2.3. Biofilm removal assay*

Biofilms of *A. baumannii* were grown in 36 wells of a 96-well microplate (test plate) by adding 50 μl sterile TSB and 50 μl of TSB inoculated with $1 \times 10^6$ CFU/ml culture. Plates were incubated at 37 °C, 140 rpm for 24 h. On a separate 96-well microplate (titration plate) a doubling dilution series was prepared with 75 μl of TSB and 75 μl of antimicrobial solution (stock concentration, 8192 μg/ml). The wells containing biofilm were washed three times with ¼ strength Ringer's solution and 75 μl sterile TSB was then added to each well. 75 μl from the titration plate, containing a specific concentration of peptide, was added to the corresponding well on the test plate. This resulted in each well containing 150 μl of TSB, which ensured the biofilm was completely submerged, and the test wells containing a doubling dilution series of antimicrobial (2048 μg/ml to 2 μg/ml). 150 μl sterile TSB was added to the negative control wells. Plates were covered and incubated in a shaking incubator for 24 h at 37 °C, 140 rpm. After incubation, the wells were emptied by tipping onto absorbent paper, washed three times with 200 μl ¼ strength Ringer's solution and air fixed for 1 h under aseptic conditions.

The percentage of biofilm removal was quantified by measuring the absorbance after applying crystal violet stain as described previously. The absorbance (at 590 nm) was determined for each sample. Biofilm reduction (%) was determined by comparison of the absorbance readings of the samples with the untreated control biofilms.

SPSS Statistics 21 software (IBM, USA) was used to assess the significance of the biofilm removal assay results. The samples t-test was applied to assess the statistical difference of 95% ($p \leq 0.05$) which was considered as statistically significant or 99% ($p \leq 0.01$) which was considered highly significant.

### *2.4. Scanning electron microscopy*

*A. baumannii* biofilms were prepared in 150 µl TSB in 96-well microplates. Calgary Biofilm Device (CBD) lids (Nunc-TSP Screening Plate Lids, ThermoScientific, UK) were placed on the plates with the polystyrene pegs protruding into the broth. Plates were sealed and incubated at 37 °C, 140 rpm for 24 h. After incubation, the pegs were washed by submerging in 200 µl of sterile ¼ strength Ringer's solution and leaving for 2 min and this process was repeated twice more. Each well was then filled with 100 µl sterile TSB.

Antimicrobial challenge plates were prepared, containing agent concentrations of 1 µg/ml, 10 µg/ml and 100 µg/ml. The CBD lid was placed onto this challenge plate, covered and incubated at 37 °C, 140 rpm for 24 h. After 24 h the pegs were washed by submerging the CBD lid in 250 µl of sterile ¼ strength Ringer's solution and leaving for 2 min and subsequently repeated twice more.

0.1 M sodium cacodylate buffer was prepared to give a pH reading of 7.2. The CBD pegs were then submerged in this solution for 3 min.

2.5% glutaraldehyde solution was prepared. The CBD lid was immersed in the buffer, covered and left at ambient temperature (21 ± 2 °C) for 40 min. The CBD lid was then removed and twice washed in distilled water for 2 min. Biofilms were then dehydrated sequentially by placing twice into 50% methanol (Fisher Scientific, UK), 70% methanol and finally 100% methanol. CBD lids were left in each solution for 2 min. Pegs were subsequently air dried for 4 days.

Pegs were removed from the CBD lid and mounted onto 0.5″ aluminium specimen stubs, fixed using carbon adhesive discs (Agar Scientific, UK). After mounting, the carbon tabs were painted with graphite and the pegs were coated with approximately 15–20 nm of platinum. *A. baumannii* biofilms on the surface of the pegs were visualised using a FEI Quanta FEG 650 Scanning Electron Microscope.

### *2.5. Atomic force microscopy*

Atomic force microscopy (AFM) was used to obtain topographic images of *A. baumannii* cells when subjected to peptide concentrations equating to ½ MBC, MBC and 2× MBC. These concentrations were 0.25, 0.5 and 1 µg/ml for colistin and 2, 4 and 8 µg/ml for both bicarinalin and BP100. 150 µl suspensions, containing inoculated TSB with *A. baumannii* cell density of $5 \times 10^5$ CFU/ml and appropriate concentrations of antimicrobial, were prepared in 96-well microplates. Plates were incubated at 37 °C, 140 rpm for 2 h.

After incubation the suspension was pipetted onto a poly-L-lysine coated slide (Sigma Aldrich, UK) and left at ambient room temperature (21 ± 2 °C) for 20 min. Slides were rinsed with distilled water and left to air dry.

Images were captured using a Bioscope Catalyst AFM (Bruker, Germany) operated in PeakForce tapping mode using ScanAsyst-air tips (Bruker, Germany). Images were acquired on an area of 4 $\mu m^2$ at a scan rate of 0.5 Hz.

### *2.6. Vesicle leakage assays*

Liposomes were made from lipids extracted from *A. baumannii*. *A. baumannii* cultured at 37 °C was harvested and a total lipid extraction conducted as described by Bligh & Dyer [27], with an extra step to extract any lipid remaining in the supernatant plus 3× washing steps with 1 M KCl. The extracted lipid was stored in chloroform at −20 °C. A Stewart lipid assay was conducted to quantify the lipid extracted.

2 mg lipid, dried from chloroform in a round-bottomed flask, was hydrated in 1 ml 100 mM 5 (6)-carboxyfluorescein (Sigma Aldrich, UK) and allowed to hydrate for 25 min with occasional shaking. The resulting suspension was extruded through a 400 nm filter and then a 100 nm Nuclepore track-etched polycarbonate membrane (Whatman, UK) using a mini-extruder (Avanti Polar Lipids, USA) at 37 °C. The extruded liposomes were then washed 3 times to remove un-encapsulated carboxyfluorescein by pelleting at 100,000 g and re-suspending in 50 mM Tris–HCl buffer and 10 mM NaCl at pH 7.3 before final re-suspension in 1 ml of the same buffer.

6.25 µl of the above liposome suspension was added to 500 µl aliquots of antimicrobial peptide solutions at the following concentrations: 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128, 256 & 512 µg/ml in the above Tris buffer. An additional concentration of 0.125 µg/ml was used for colistin due to its lower MIC. 100 µl aliquots were transferred to black 96-well microplates for fluorescence measurements at 490 nm excitation and 520 nm emission using an Infinite 200Pro (Tecan, Switzerland). Carboxyfluorescein leakage was measured relative to the same quantity of liposomes suspended in 1% SDS following approximately 30 min exposure to the agent.

## 3. Results

### *3.1. Antimicrobial assays*

MIC and MBC values were determined to be 0.5 µg/ml (0.43 µmol/L) for colistin, 4 µg/ml (1.8 µmol/L) for bicarinalin and 4 µg/ml (2.8 µmol/L) for BP100 (data not shown). Fig. 1 shows that the MBIC was found to be similar to the MIC and MBC for each agent with 90% of biofilm formation inhibited at 0.5 µg/ml colistin

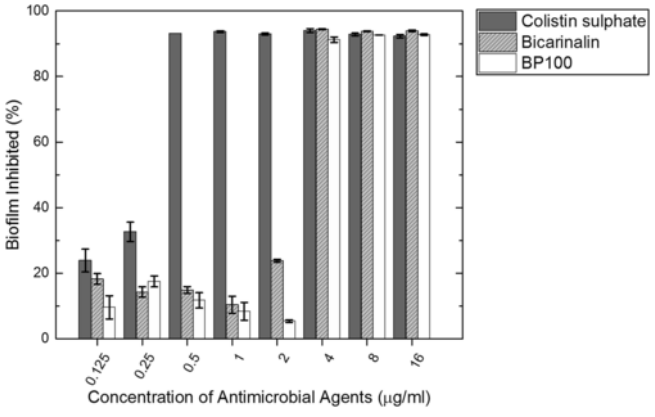


**Fig. 1.** MBIC for colistin sulphate, bicarinalin and BP100. *A. baumannii* was subjected to a doubling dilution series (2–2048 µg/ml) of antimicrobial for 24 h. After washing and air fixing for 1 h, samples were stained with 1% crystal violet. After 10 min the stain was solubilised with 96% ethanol and the absorbance at 590 nm was read against a 96% ethanol blank. The percentage biofilm inhibited was calculated by comparing against an untreated bacterial control. The experiment was carried out three times in triplicate.

and 4 μg/ml bicarinalin and BP100. This also showed that at concentrations below the peptides' respective MBCs, biofilm formation was possible with only 36% biofilm inhibition for colistin, 23% for bicarinalin and 20% for BP100. Fig. 2 shows the eradication of a 24-h biofilm when subjected to increasing concentrations of peptide. At 1 μg/ml of colistin, 27% of biofilm was removed. As the peptide concentration increased, more biofilm was eradicated with 52% removed at 512 μg/ml. Bicarinalin removed 18% at 1 μg/ml which then increased to 70% at 128 μg/ml; no further biofilm eradication was observed with higher concentrations. BP100 removed 17% at 1 μg/ml which remained low until 16 μg/ml. Above this concentration, the biofilm removed increased from 27% at 32 μg/ml to 76% at 512 μg/ml, higher than both colistin and bicarinalin, to 93% at 2048 μg/ml.

### *3.2. Microscopy*

SEM (Fig. 3) and AFM (Fig. 4) provide visualisation of the effects of these agents on *A. baumannii* cells. Fig. 3 demonstrates that, as the concentration of the agents increases, the morphological changes to the cells become more pronounced including evidence of blebbing and a more variable cell shape with shrinkage and membrane disruption. The coverage across the surface also reduces: with no addition of antimicrobial agent the cells are packed closely together and cover the whole interface; as the peptide concentration increases visible gaps are seen between clusters of cells. The cellular morphological changes are also observed in Fig. 4 by AFM. Blebbing is observed, causing varied cell shape and shrinkage at the MBC concentrations, and the cells differ greatly compared to the cocci-bacilli shaped cells seen with no antimicrobial treatment and below the MBC.

### *3.3. Vesicle leakage assays*

To determine the mechanism of action of the antimicrobial agents, a vesicle leakage assay was performed. Liposomes produced from lipids extracted from *A. baumannii* (Fig. 5) all leaked carboxyfluorescein in the presence of the antimicrobials. The concentration of agent causing half-maximal leakage for each agent was ~1.75 μg/ml for colistin, ~2.75 μg/ml for BP100 and ~2 μg/ml for bicarinalin (given maximal leakage of 70%, 65% and 56% respectively). Vesicle leakage results support the proposal that these agents exert effects on the bacterial cell membrane causing significant disruption to the cell surface (as shown in Fig. 4) and eventually cell lysis (as shown in Fig. 3).

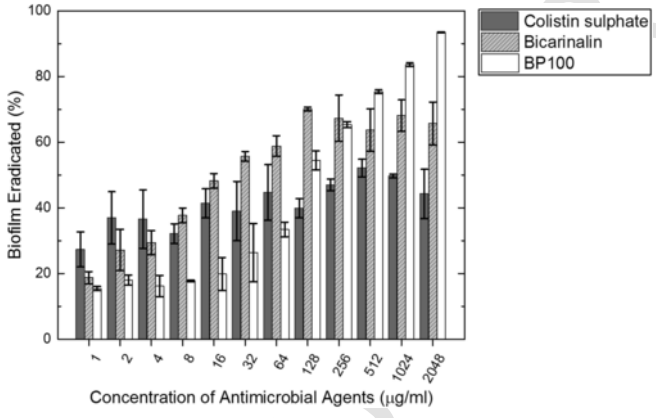


**Fig. 2.** Percentage biofilm eradication by colistin sulphate, bicarinalin and BP100. *A. baumannii* biofilms were grown for 24 h and then subjected to a doubling dilution series (2–2048 μg/ml) of antimicrobial for 24 h. After washing and air fixing for 1 h, samples were stained with 1% crystal violet. After 10 min the stain was solubilised with 96% ethanol and the absorbance at 590 nm was read against a 96% ethanol blank. The percentage biofilm eradicated was calculated by comparing against an untreated bacterial control. The experiment was carried out twice in triplicate.

## 4. Discussion

*A. baumannii* susceptibility to colistin was similar to that determined by Li et al. [28] and Sauger [29], confirming that *A. baumannii* 19,606 strain is colistin susceptible as defined by The Clinical and Laboratory Standards Institute. Bicarinalin results were compared with those of Rifflet [18] and Téné [19] who investigated *Cronobacter* spp., *Enterobacter* spp. and *Staphylococcus* spp., Results suggest that *A. baumannii* is generally more susceptible to bicarinalin than these other bacteria. Antimicrobial activity results for BP100 against *A. baumannii* 19,606 were comparable to other bacteria in previous studies [20,21].

Although the MBC for both bicarinalin and BP100 was 4 μg/ml, the molar concentration of peptide differed (1.8 μmol/L for bicarinalin and 2.8 μmol/L for BP100). This is primarily due to the differences in molecular weight (bicarinalin 2213.78 g/mol, BP100 1420.88 g/mol) indicating that, although BP100 is smaller in length, it exerts a similar inhibitory and potent effect on *A. baumannii*. By comparison, colistin has a MBC of 0.43 μmol/L (with the smallest molecular weight, 1155.4 g/mol).

MBIC results demonstrated that, at the MBC for all three antimicrobials studied, there was less than 10% biofilm formed compared to the control. Additionally, at concentrations of antimicrobial agents equal to ½ MBC (2 μg/ml for bicarinalin and BP100, 0.25 μg/ml for colistin) there was less inhibition of biofilm formation with 77% of biofilm formed in the presence of bicarinalin, 95% in BP100 and 64% in colistin. This highlights that even below their respective MBCs, bicarinalin and colistin have some potential to prevent biofilm formation but BP100 had very little, possibly due to differences in size and mechanism of action.

Biofilm eradication results indicated that bicarinalin is superior at eradicating *A. baumannii* biofilms than colistin above its MBC and both bicarinalin and BP100 are more effective at concentrations above 128 μg/ml. Colistin was more effective at removing *A. baumannii* biofilms at low concentrations; this was as expected due to its lower MBC which is nearly an order of magnitude less than bicarinalin and BP100. However, the maximum biofilm removed was only ~50% at 512 μg/ml. Bicarinalin was the most effective between 8 μg/ml, where it removed ~40% of the biofilm, and 128 μg/ml, where it eradicated 70% of the biofilm. Any further increase of concentration had no additional significant effect on the biofilm. The removal activity of BP100 started slowly and at 16 μg/ml it had removed only 20% of the biofilm, half that of colistin. Above 16 μg/ml, BP100 increased relatively linearly to exceed bicarinalin activity above 512 μg/ml. At 2048 μg/ml, the highest concentration tested, BP100 had eradicated ~95% of the biofilm. Colistin had the most biofilm removal activity, as expected, at low concentrations of 1–4 μg/ml, below the MBC of bicarinalin and BP100. Bicarinalin was the most active between 8 and 128 μg/ml and BP100 was the most effective from 256 to 2048 μg/ml. The superior performance of bicarinalin, and particularly that of BP100 on biofilms at higher concentrations, was not anticipated especially considering the MBC is significantly higher than colistin.

The SEM has been widely used to image bacterial biofilms [30,31], revealing, with increasing concentrations of peptide, progressive changes in cellular shape and structure of the biofilm. Membrane protrusions between bacterial cells were seen in the control sample

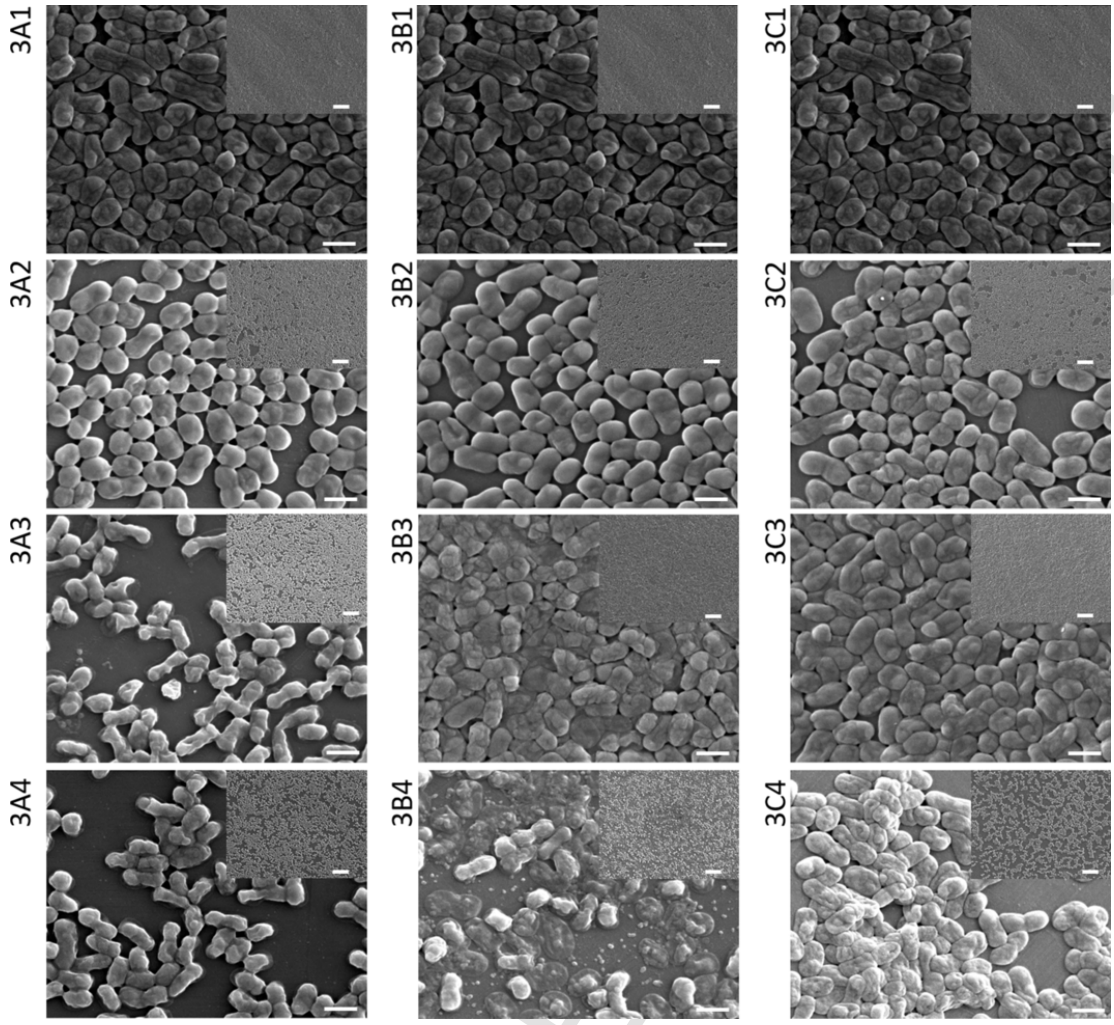


**Fig. 3.** SEM Images of *A. baumannii* biofilms. Biofilms were grown for 24 h then treated for a further 24 h with colistin (A), bicarinalin (B) and BP100 (C). Control images, no antimicrobial (1), 1 μg/ml (2), 10 μg/ml (3), 100 μg/ml (4). Scale bars on main images are 1 μm, 10 μm on inset images.

and at 1 μg/ml concentrations for each agent in this study. Pili or cellular filaments between cells have been previously documented between cells within biofilms [32,33]. At the higher concentrations of 10 μg/ml and 100 μg/ml, these protrusions were not visible suggesting the inability of the cells to form pili at these concentrations [34]. Fig. 3 clearly shows that as the concentration of agent is increased, the cell shape becomes less regular and the surface coverage is decreased.

The effect of the antimicrobials on *A. baumannii* was visualised by AFM. Fig. 4 highlights that, below the peptide MBC, the coccus-bacillus cell shape is conserved. However, at concentrations at and above the MBC, the cell surface, conformation and size are drastically affected. At the highest concentrations tested there is evidence of severe disruption to the cell surface, cytoplasmic leakage and lysed cells.

Membrane disruption caused by peptide activity is noted from various studies [20,35–37]. In this study, indentations and pores are seen in the bacterial surface with resulting cytoplasmic leakage and debris, suggesting loss of turgor pressure [38]. As the peptide concentration increased, more intense membrane perturbation and cytoplasmic leakage were visualised. Similar observations were described by Li et al. [35].

Although AFM imaging of bacterial cells in air is a widely used technique [20,39], it can lead to dehydration of the cell and changes in the cell surface that were not directly caused by peptide activity. However, all images have been compared to the control of untreated cells.

The dye leakage assay reflects the relative potency of these peptides against *A. baumannii*, suggesting that the peptides mediate their effects through the formation of pores in the membrane although it likely reflects planktonic growth more accurately than biofilm-like structures. It cannot be directly concluded whether the peptides exert their effects on the cytoplasmic or outer membrane of the cell. The visualised bacterial cell morphological changes seen in SEM (Fig. 3) and AFM (Fig. 4) images are likely to relate to outer membrane disorder while the vesicle leakage assay may represent disruption to the cytoplasmic membrane.

Overall the results support the proposal that bicarinalin and BP100 could be used to control infections caused by biofilm-producing prokaryotes. However, Torcato et al. [40] observed 50% cytotoxicity against HELA cells at 49.2 ± 1.4 μmol/L for BP100 which is higher than the MBC observed here (2.8 μmol/L) but lower than the concentrations needed for antibiofilm activity, suggesting that clinical antibiofilm activity of BP100 may be unlikely. Colistin performs well against planktonic cells of *A. baumannii* but significantly less well

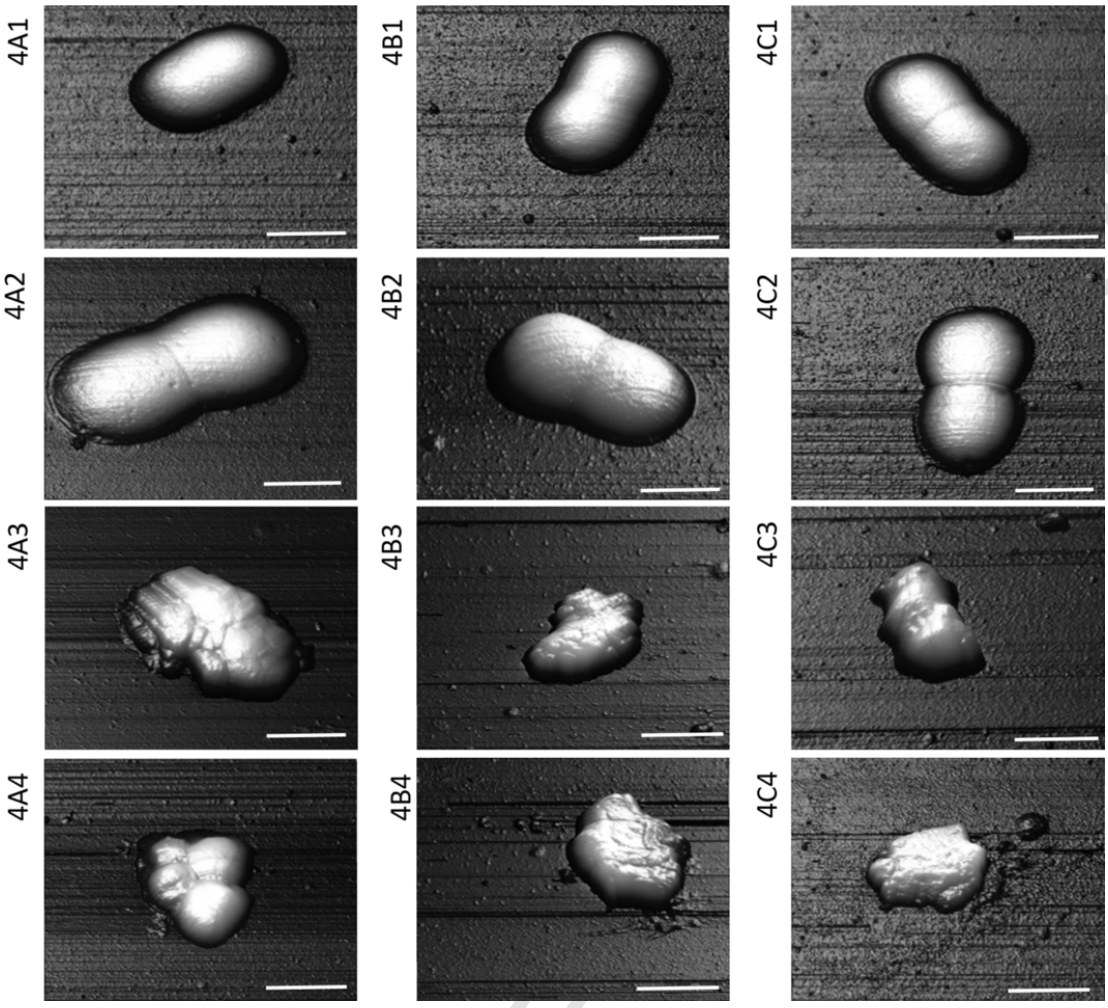


**Fig. 4.** AFM Images of *A. baumannii* cells after being treated with colistin (A), bicarinalin (B) and BP100 (C) for 2 h and fixed onto a glass slide coated with poly-L-lysine. Control images, no antimicrobial (1), 1/2 MBC (2), MBC (3), 2× MBC (4). Images were 4 $\mu m^2$ and 256 lines at 0.5 Hz. Scale bars are 1 μm.

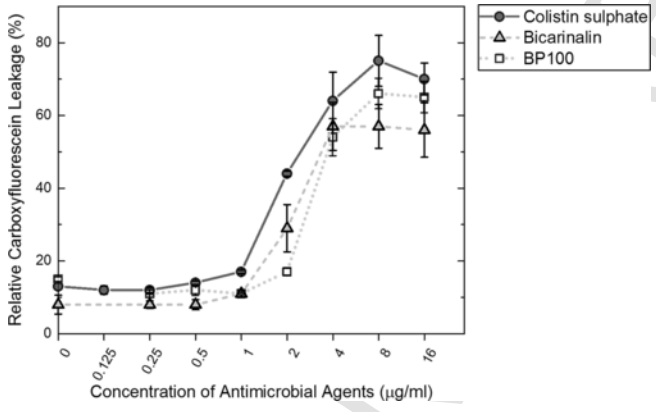


**Fig. 5.** The relative leakage of carboxyfluorescein from liposomes made from *A. baumannii* total lipid extract in the presence of increasing concentrations of the three antimicrobial agents. Error bars show the standard error around the mean of 3 replicates.

against biofilms. As *A. baumannii* is able to form biofilms, it is essential that investigations into the efficacy of novel agents are performed rigorously on biofilms.

The results indicate that bicarinalin and BP100 both have similar bactericidal and generally better anti-biofilm activity against *A. baumannii* than colistin, with evidence supporting the idea that these peptides mediate their effects through the formation of pores in the membrane. This is encouraging but cytotoxic studies will determine whether, at these concentrations, bicarinalin and BP100 can be used in clinical treatment.

## Conflict of interest

There is no conflict of interest between any author and any other people or organisation that could inappropriately influence this work.

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